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New cytochrome P450 1B1, 1C1, 2Aa, 2Y3, and 2K genes from Chinese rare minnow (*Gobiocypris rarus*): Molecular characterization, basal expression and response of rare minnow CYP1s and CYP2s mRNA exposed to the AHR agonist benzo[a]pyrene

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HIGHLIGHTS

- New CYP1B1, CYP1C1, CYP2K, CYP2Aa, and CYP2Y3 gene from rare minnow were cloned.
- Basal expression patterns of the CYPs varied in these tissues of rare minnow.
- High induction of CYP1 transcripts by benzo[a]pyrene was observed in rare minnow.
- The fish CYP2Y3 mRNA can be induced by the AHR agonist benzo[a]pyrene.

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ABSTRACT

Cytochrome P450 (CYP450) genes play an important role in catalyzing oxidative metabolism of toxicants. Recently, CYP1 subfamily were discovered and reported in fish, however, little is known regarding the CYP2 isoforms in fish. In the present study, the cDNA fragments of CYP 1B1 and 1C1 and CYP2Aa, 2Y3, and 2K of rare minnow were cloned and exhibited a high amino acid sequence identity compared with their zebrafish orthologs. Basal expression showed CYP1C1 and CYP 2Aa expression were observed in all eight tissues analyzed (liver, gill, intestine, kidney, spleen, brain, skin, and muscle). CYP 1A, and 1B1 expression was found in all tissues except for muscle and skin. However, CYP 2Y3 was expressed in liver, spleen, intestine and muscle whereas CYP 2 K in liver, kidney and intestine. 4 and 100 μg L^{-1} Benzo[a]pyrene (BaP) induced patterns showed that CYP 1A, 1B1 and 1C1 expression in liver, gill, and intestine was strongly up-regulated ($p < 0.05$). Furthermore, CYP 2Y3 was strongly induced in liver from BaP treatments ($p < 0.05$). The high induction on mRNA level of CYP1s and CYP 2Y3 by BaP could be associated with catalyzing detoxification and indicated that CYP2s may also be potential biomarker to screen AHR agonist. The high responsiveness of CYP1 and 2 genes suggested Chinese rare minnow is feasible to screen and assess pollution with AHR agonist.

1. Introduction

Cytochrome P450 enzymes are a large superfamily of heme-proteins, which are involved in the phase I biotransformation of many endogenous and exogenous compounds. About 12,456 CYP450s have been identified and found in animals, plants, fungi bacteria, and viruses (Nelson, 2011). In mammals, the CYP1-4 isoforms are mainly involved in the metabolism of xenobiotics such as drugs, chemical carcinogens (Goldstone et al., 2010). CYP genes are regulated by the constitutive androstane receptor (CAR), pregnane × receptor (PXR), hepatocyte nuclear factor 4 (HNF-4), and aryl hydrocarbon receptor (AHR) (Xu et al., 2005; Monostory and Pascussi, 2008). In the previous studies, CYP1A, 1B1, 1C1, 1C2, and 1D1 genes in fish were discovered and identified (Zanette et al., 2009). Several studies have showed the CYP1 genes are regulated by AHR in mammals as well as in fish (except fish CYP1D1) (Shimada et al., 2002; Lin et al., 2003; Gao et al., 2011). A number of isoforms of AHR including AHR1a, AHR1b, and AHR2 in fish were reported, whereas AHR2 has been proved to regulate the expres-

Abbreviations: CYP450, cytochrome P450; AHR, aryl hydrocarbon receptor; BaP, benzo[a]pyrene; PAHs, polycyclic aromatic hydrocarbons.

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sion of CYP1A in fish (Prasch et al., 2003; Timme-Laragy et al., 2007).

The CYP2 family is the largest and most diverse of the vertebrate CYPs, and plays an important role in mammalian drug metabolism (Goldstone et al., 2010; Kirischian et al., 2011). 16, 17, 27, and 47 CYP2 isforms were reported in human, rabbit, oposum, and zebrafish, respectively (Kirischian et al., 2011). In mammals, CYP2S1 is induced by dioxin and the induction is mediated by the AHR. Full complement of CYP genes were identified only in zebrafish (Goldstone et al., 2010). The CYP 2X1 in channel catfish (Mosadeghi et al., 2007); CYP 2M1 and CYP 2K1 in rainbow trout (Yang et al., 1998); CYP 2Ps and CYP 2N in killifish (Yang et al., 1998); and CYP 2Y3, CYP 2C33-like and CYP 2P1-like in Atlantic cod (Olsvik et al., 2009) were identified. Phylogenetic tree analysis showed that CYP 2Y3 and CYP 2Y4 of zebrafish shared synteny with a cluster of CYP2 genes including CYP2S1 of human, whereas function of CYP2Y3 and CYP2Y4 in fish is unknown (Goldstone et al., 2010). Though some CYP2s were discovered and identified in fish, their function, tissue distribution, and induced patterns were not well-characterized.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic contaminants arising from incomplete combustion or pyrolysis of organic material. Exposure to PAHs has been associated with a variety of toxicity, including developmental, immunological disorders, mutagenesis and carcinogenesis (Nicol et al., 1995; De Jong et al., 1999; Timme-Laragy et al., 2007). It is widely held that the understanding of the toxicity caused by PAHs requires the study of AHR and its downstream genes including the CYP450 enzymes (Matsumoto et al., 2007). In addition, a number of studies have linked CYP1A induction with PAH metabolites, DNA adducts, and tumor formation in fish (Wang et al., 2010), and the CYP1 genes are involved in carcinogen bioactivation of PAHs including benzo[a]pyrene (BaP) (Ghanayem et al., 2000). BaP is one of the most studied environmentally relevant PAHs, and has been considering as an AHR agonist based on its ability to bind the receptor. BaP is most often identified with cancer due to being a well-established chemical mutagen (Thompson et al., 2010). Based on this knowledge, BaP is suitable as a reference chemical to study responses of CYPs in fish.

Most previous studies have reported that induced patterns of CYP1s in fish including zebrafish (Jönsson et al., 2007), killifish (Zanette et al., 2009), and three-spined stickle blacked (Gao et al., 2011) exposed to PCB126. Though CYP1A gene of rare minnow were identified in previous studies (Liu et al., 2008), the molecular characterization and basal expression and induced expression of other CYP1s, especial CYP2s, were not investigated. The aim of present study was to identify CYP 1B1, 1C1 and CYP2Aa, 2Y3, 2K genes in rare minnow and to characterize their basal expression pattern in eight tissues (liver, gill, intestine, kidney, spleen, brain, skin, and muscle) and, to investigate the BaP induced effects of the CYPs in liver, gill, and intestine of rare minnow, as these tissues (liver, gill, and intestine) are mainly involved with the uptake and metabolism of pollutants (Costa et al., 2012).

The rare minnow is distributed mostly in the upstream region of the Yangtze River and in the Sichuan Province of China. It is considered to be an appropriate species for the assessment of endocrine disrupting chemicals due to its small size, ease of culture, short life cycle and prolific egg production with high fertilization and hatching rates (Zha et al., 2007; Li et al., 2009; Yang et al., 2010).

2. Materials and methods

2.1. Chemicals

BaP (purity >98%) and acetone were purchased from Sigma (Chemical Co., USA). Stock solution of BaP was prepared by dilution in acetone.

2.2. Test fish and culture conditions

The brood stock of rare minnow was raised in a flow through system with dechlorinated tap water (pH: 7.2–7.6; hardness: 44–61 mg $CaCO_3$ L^{-1}; and temperature: 25 ± 1 °C) with a photo period of 16:8 h (light:dark) and has been used for testing chemicals in our laboratory for more than 10 years (Zha et al., 2007; Li et al., 2009). Fish were fed a commercial food pellet (Trea, Germany) at a rate of 0.1% body weight per day and newly hatched brine shrimp (*Artemia nauplii*) two times daily.

2.3. Cloning of cDNA fragments of CYP 1B1, 1C1, 2Aa, 2Y3, and 2K by RT-PCR

Total RNA was isolated from the liver of rare minnow using SV Total RNA Isolation System following the manufacturer's protocol (promega, USA). Then RNA samples were dissolved in ribonuclease-free water and stored at −80 °C until the process of reverse-transcriptase polymerase chain reaction.

The protocol of reverse-transcriptase polymerase chain reaction is according to previous report of our laboratory (Li et al., 2009). The reverse transcription reaction mixtures containing 10 μL of total RNA, 2 μL (0.05 μg μL^{-1}) of $Oligo(dT)_{15}$, and diethylpyrocarbonate-treated water (a total volume of 12 μL), were heated to 70 °C for 5 min and quickly chilled on ice. After cooling, 50 mM Tris–HCl buffer (pH 8.3), 75 mM KCl, 10 mM dithiothreitol, 3 mM $MgCl_2$, 2 mM deoxy-nucleotide triphosphate (10 mM each), 40 units of RNAasin (RNAase inhibitor; Promega), and 200 U of Moloney Murine Leukemia Virus Reverse Transcriptase (Promega) were added to a total volume of 25 μL and incubated for 1 h at 37 °C. Thereafter, the reaction mixture was heated to 70 °C for 10 min to inactivate the reverse transcription.

To obtain cDNA fragments of the CYP genes (CYP1B1, 1C1, and 2K), the primer pairs were designed based on highly conserved regions according to known sequences of other fish species available in GenBank; and the primer pairs of CYP 2Aa and 2Y3 were designed based on zebrafish CYP 2Aa and 2Y3 sequences, respectively (Table 1). The detailed information of the PCR experiment was as follows, each 50 μL DNA amplification reaction contained polymerase chain reaction buffer (20 mM Tris–HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$), 200 μmol dNTP, 20 pmol of each gene specific primer, 1 μL of a 20-fold dilution of total cDNA, and 2.5 U Taq DNA polymerase (Invitrogen). The thermocycle program included a denaturation step at 94 °C (8 min); 35 cycles of 94 °C (30 s), 55 °C (30 s), and 72 °C (30 s); and a final elongation step at 72 °C (10 min). The amplification PCR products were analyzed by 1.5% agarose gel electrophoresis. The bands of expected size were excised and purified using an Agarose Gel DNA Purification Kit Version 2.0 (TaKaRa). The isolated fragments were inserted into pMD 19-T Vector (TaKaRa) and transformed into *Escherichia coli* (DH 5α). The partial cDNAs were sequenced and compared by BLAST to the sequences available in GenBank.

2.4. The experimental design for exposure and tissue distribution of the CYPs

Healthy rare minnows (n = 72), about 4 month old and the offspring from the same pair of brood stock, were used in this experiment. The body weights and lengths were 0.55 ± 0.17 g and 37.23 ± 3.25 mm, respectively. Reproductively immature fish were used to minimize the effects of gender and reproductive state differences on the expression of CYP450 enzymes (Willett et al., 1997; Barber et al., 2007). The fish were exposed to various concentrations (4, 100 μg L^{-1}) of BaP. A vehicle treatment containing acetone served as a control (Jönsson et al., 2007), the ratio of vehicle to water was 1:10,000 (v/v).

Table 1
Primer sequences used for cloning of CYP 1B1, 1C1, 2Y3, 2K, and 2Aa, and quantification of CYP1 and CYP2 genes expression by real-time PCR in rare minnow.

Gene	Sequence (5′ → 3′)	Product size (bp)	Genbank accession no.
Cloning			
CYP1B1	F: CCACAGCCAACATCCAGA	239	KC136245
	R: CATCCAGGGCATCACATC		
CYP1C1	F: ATTGAGCACGGGAAAGAG	232	KC136246
	R: GGACGAAACTGGTGAAGC		
CYP2K	F: ACATTGTGCCCGTTGAGTC	266	KC136247
	R: AGTTGTGAAAGCGGTAGCT		
CYP2Aa	F: TCGGGTGTTAGAGGAAAGC	247	KC136248
	R: CTCATTGGCGTTCTGTTTGA		
CYP2Y3	F: TAGACATCACGCCATCTTAAG	367	KC165029
	R: CGTCAGTGAAGGGAAGAGACT		
Real-time PCR			
β-actin	F: CAGGGCGTGATGGTGGGGAT	226	DQ539421
	R: GGTTGGCTTTGGGGTTGAG		
CYP1A	F: ATCATCGGAAATGTGCTGG	170	EU106660.1
	R: GAGAACTCCTCGCCCTGTT		
CYP1B1	F: TTCTCCACAGCCAACATC	138	
	R: CGACACCACTAAATACCG		
CYP1C1	F: CGGGACAGGACACGATGT	157	
	R: TCCAGGTATGCGAGGTTG		
CYP2Aa	F: TGTTTGGAGATCGCTTCG	167	
	R: ATTGGCGTTCTGTTTGAC		
CYP2K	F: AATGGGAGAAACCAAACA	163	
	R: TGAAGGAGGGAAGTAAAGA		
CYP2Y3	F: CCAGAGACCACAGCACAACT	156	
	R: GCGTCAGTGAAAGTAGGGACT		

The fish were acclimated to the exposure aquarium (3 L) for 7 days before initiate of the experiment. Then, the fish were randomly distributed into 3 experimental groups, each group contained 3 replicate aquariums and each replicate aquarium included 8 fish. During the experiment, water temperature was maintained at 25 ± 1 °C and pH at 7.0 ± 0.2. Fish were fed two times a day with newly hatched brine shrimp. After 12 days exposure, the fish were sacrificed and the tissues (liver, gill and intestine) were excised and immediately frozen in liquid nitrogen and stored at −80 °C.

To determine tissue distribution of the CYP genes, another 15 immature fish (4 month old) were used, and the fish were randomly distributed into 3 groups. Thereafter, the fish were sacrificed and the tissues (liver, gill, intestine, kidney, spleen, brain, skin, and muscle) were excised from each group (containing five replicates), and immediately frozen in liquid nitrogen and stored at −80 °C.

2.5. Determining the CYP genes expression by real-time PCR

To determine tissue distribution of the CYPs and assess their transcriptional effects following BaP exposure, real-time PCR was performed in a Mx3005P real-time quantitative polymerase chain reaction system (Stratagene, USA). The PCR reaction mixtures (25 μL) consisted of Brilliant II SYBR Green QPCR master mix, 300 nM forward primer and 300 nM reverse primer. The primer pairs used for real-time PCR were shown in Table 1. For each sample, gene expression was analyzed in triplicate with the following protocol: 95 °C for 8 min and 40 cycles of 30 s at 95 °C, 30 s at 57 °C and 30 s at 72 °C. Melt curve analysis was performed on the PCR products at the end of each PCR run to ensure that a single product was amplified.

The basal mRNA expression of the CYP genes was calculated by the $2^{-\Delta Ct}$ method (Schmittgen and Livak, 2008) using β-actin as the reference gene. For fold-change in expression after exposure the $2^{-\Delta\Delta Ct}$ method was used. The mean value of these triplicate measurements were used for calculations of mRNA expressions.

2.6. Statistical analysis

Statistical analyses were performed with the SPSS (version 13.0) and OriginPro (version 8.0). All quantitative data are expressed as the mean ± S.E. of the mean (S.E.M.). The differences among transcript levels of the CYPs in different tissues, and the means between control and BaP exposure groups were evaluated using one-way ANOVA ($p < 0.05$), followed by Dunnett's test for multiple comparisons.

3. Results

3.1. Cloning and analysis of cDNA fragments of CYP 1B1, 1C1, 2Aa, 2Y3, and 2K

The CYP 1B1, 1C1, 2Aa, 2Y3, and 2K partial cDNAs were isolated and cloned from rare minnow. The partial fragments of these CYPs have been sequenced and submitted to GenBank. Accession numbers are KC136245 for CYP1B1, KC136246 for CYP1C1, KC136248 for CYP2Aa, KC165029 for CYP2Y3, and KC136247 for CYP2K.

Sequence alignment and phylogenetic analysis revealed the predicted amino acid sequences of the CYP1s in rare minnow have high similarities with those of other fish and mammalian species (Fig. 1). For three CYP2s, the partial CYP2K amino acid sequences display high identity with other sequences of fish CYP2Ks (Fig. 1). CYP2Y3 and CYP2Aa share homology with mammalian CYP2Cs and CYP2Js, respectively (Fig. 1). Moreover, the corresponding CYP2s in rare minnow and zebrafish display high degrees of amino acid sequence identity. The predicted amino acid sequences of rare minnow share 86%, 87%, , 87%, 75%, and 91% pair-wise identity with CYP1B1, CYP1C1 , CYP2Aa2, CYP2Y3, and CYP2K19 of zebrafish, respectively (Fig. 1).

3.2. Tissue distribution pattern of CYP1 and CYP2 isoforms

The tissue distribution patterns of CYP 1A, 1B1, 1C1, 2Aa, 2Y3, and 2K in eight different tissues (liver, gill, intestine, kidney, spleen, brain, skin, and muscle) in immature rare minnow were assessed by real-time RT-PCR.

The results showed different tissue expression patterns for these CYPs (Fig. 2). Expression of CYP1C1 and 2Aa was observed in all eight organs, both CYP1A and CYP1B1 transcripts were detected in all the tissues except for muscle and skin (Fig. 2). However, the expression of CYP 2Y3 and 2K was observed only in four organs (liver, muscle,

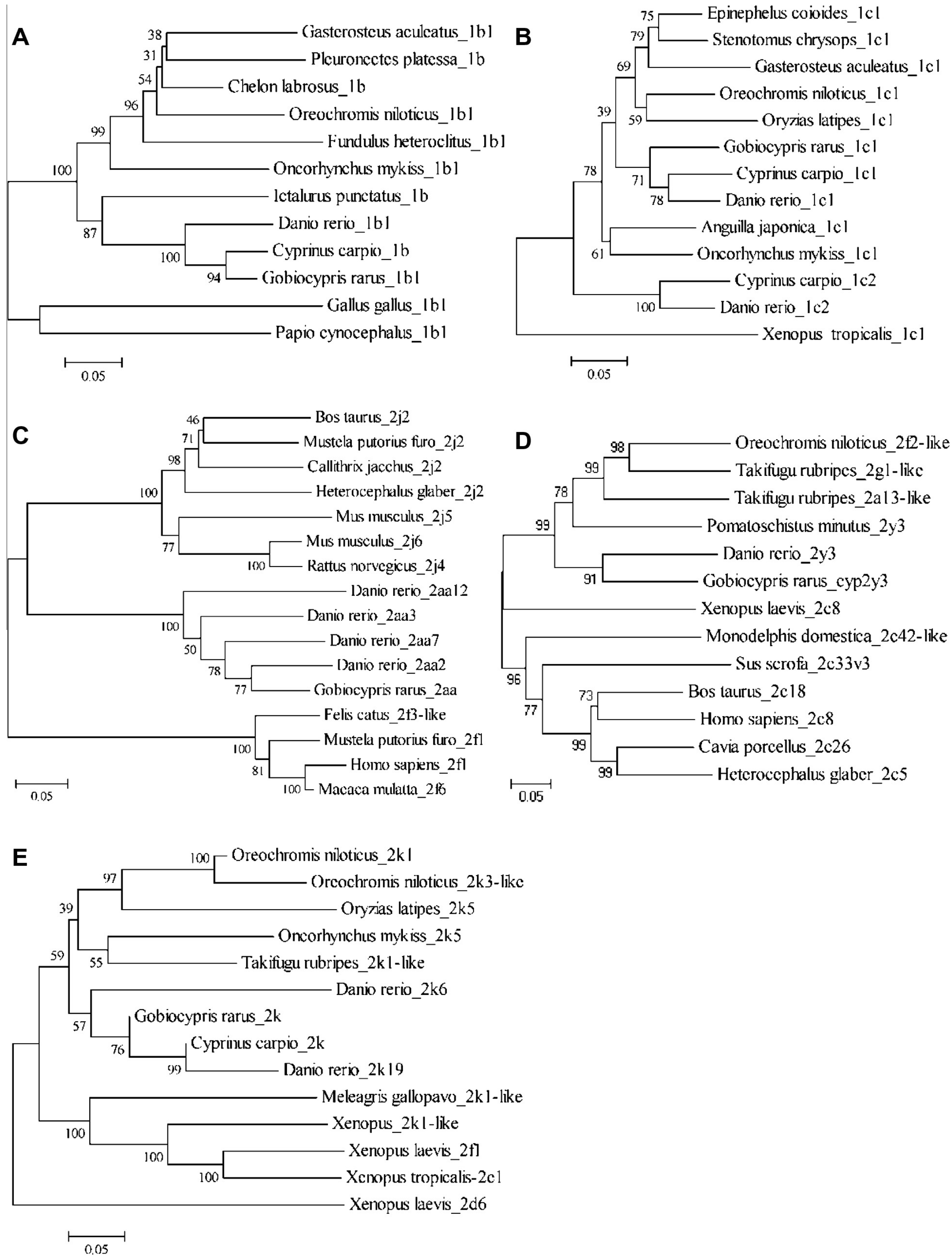

Fig. 1. Phylogenetic tree showing the relationships of rare minnow (A) CYP1B1, (B) CYP1C1, (C) CYP2Aa, (D) CYP2Y3, (E) CYP2K from other vertebrates. The phylogenetic analysis was carried out by MEGA 5 on deduced amino acid sequences. Sequences were obtained from the NCBI GenBank database. Branch length is proportional to estimated divergence across each branch. One thousand bootstrap replicates were performed and are expressed as percentages noted on each node.

intestine, and spleen) and three organs (liver, kidney, and intestine), respectively (Fig. 2). The expression of all six CYP genes (CYP 1A, 1B1, 1C1, 2Aa, 2Y3, and 2K) was observed in liver and intestine, and all these genes transcription was detected in the kidney except the CYP2Y3. CYP1A shows the highest and lowest levels of mRNA expression in the liver and brain, respectively. However, CYP1B1 shows the highest level of mRNA expression in the brain, but the expression was low in liver. Expression level of CYP1C1 was much higher in muscle and skin than the other organs. For the CYP2 isoforms, the highest levels of CYP2Y3, 2K, and 2Aa transcripts were observed in spleen, liver, and intestine, respectively (Fig. 2).

The basal levels of the six CYP1 and CYP2 genes were also compared within a tissue (liver, gill and intestine) and data are presented as a percentage of the highest CYP transcript in each tissue (Fig. 3). The liver showed a significantly higher expression of CYP2 than of the CYP1 genes (100%, 24.07%, 21.46% of CYP2Y3, 2Aa, and 2K, respectively; 12.46%, 5.74%, 1.08% of CYP1C1, 1B1, and 1A, respectively). In the liver, CYP2Y3 was most highly expressed and CYP1B1 showed a weak expression. CYP1C1 was highly expressed, whereas, the expression of CYP 2Y3 and 2K was not detected in gills of rare minnow. In the intestine, CYP2K was much more highly expressed than other CYPs (Fig. 3).

3.3. Transcriptional effects of CYP1 and CYP2 isoforms following BaP exposure

The expression of CYP1A, 1B1, 1C1, 2Aa, 2Y3, and 2K in liver, gill, and intestine of rare minnow were determined after 12 days exposure to BaP (4 and 100 $\mu g\,L^{-1}$). The CYP1A, 1B1, and 1C1 expression from BaP treatments significantly increased compared with the control (acetone) in three tissues ($p < 0.05$), and the induction pattern varied in these tissue. For CYP1A, the highest induction was observed in the liver and gill (33- and 14-fold versus the control, Fig. 4). CYP1B1 showed highest induction in intestine (27-fold), while CYP1C1 showed highest induction in gill (19-fold).

For three CYP2 genes, CYP2Y3 only in liver was significantly induced from 4 and 100 $\mu g\,L^{-1}$ BaP treatments compared with the controls ($p < 0.05$) (Fig. 5). The level of CYP2Y3 induction in liver by 4 and 100 $\mu g\,L^{-1}$ BaP were 2.6- and 6-fold over the control, respectively. However, no significantly induction of CYP2Aa, and CYP2K in all three tissues from BaP treatments was observed compared with the controls (Fig. 5).

4. Discussion

4.1. Identification of new CYP 1 and CYP 2 genes in rare minnow

In our study, five previously unreported CYP genes in rare minnow were cloned and identified. The partial fragments of these CYPs have been sequenced and submitted to GenBank. Based on sequence and phylogenetic analysis of their predicted amino acid sequences, the new transcripts were classified as CYP1B1, CYP1C1, CYP2 Aa, CYP2Y3, and CYP2K.

In general, the predicted amino acid sequences of the CYP1s exhibited high homology with the same regions of other fish and mammalian species (Zanette et al., 2009; Jönsson et al., 2010). The CYP2 family is the largest and most diverse of all CYP families (Goldstone et al., 2010). Compare to the CYP1 family, relatively little is known regarding CYP2 isoforms in fish (Mosadeghi et al., 2007; Smith, 2009). In the present study, we have identified three CYP2 genes in rare minnow, and the corresponding CYP2s in rare

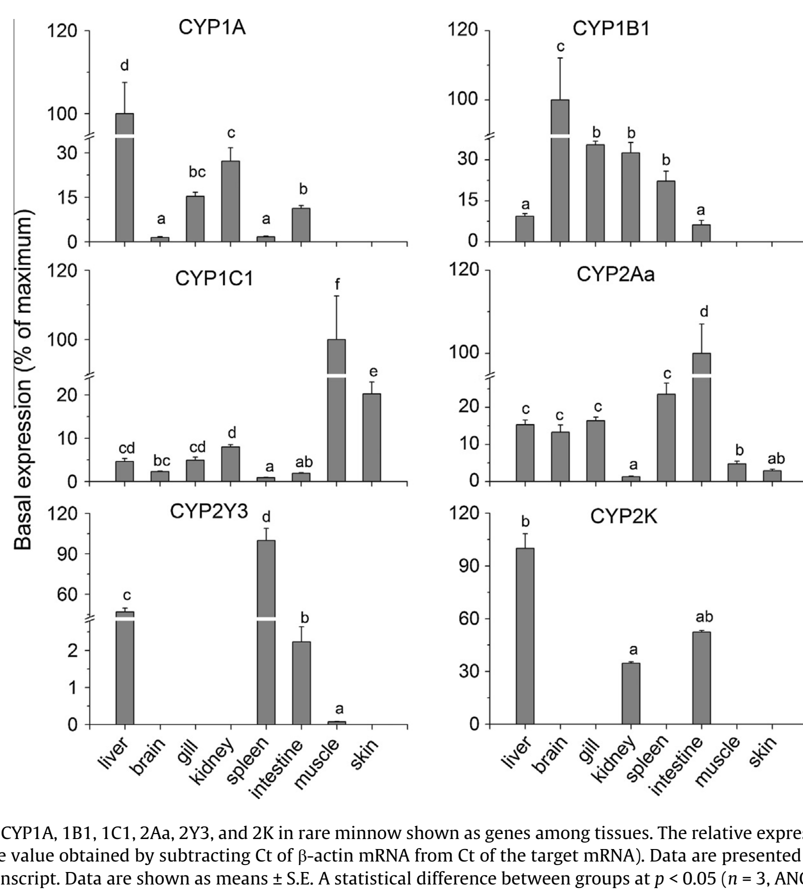

Fig. 2. Relative basal expression of CYP1A, 1B1, 1C1, 2Aa, 2Y3, and 2K in rare minnow shown as genes among tissues. The relative expression of each mRNA was calculated by the $2^{-\Delta Ct}$ method (where ΔCt is the value obtained by subtracting Ct of β-actin mRNA from Ct of the target mRNA). Data are presented as a percentage of the tissue with the highest level of a particular CYP transcript. Data are shown as means ± S.E. A statistical difference between groups at $p < 0.05$ ($n = 3$, ANOVA) is indicated by differences in the letters above the bars.

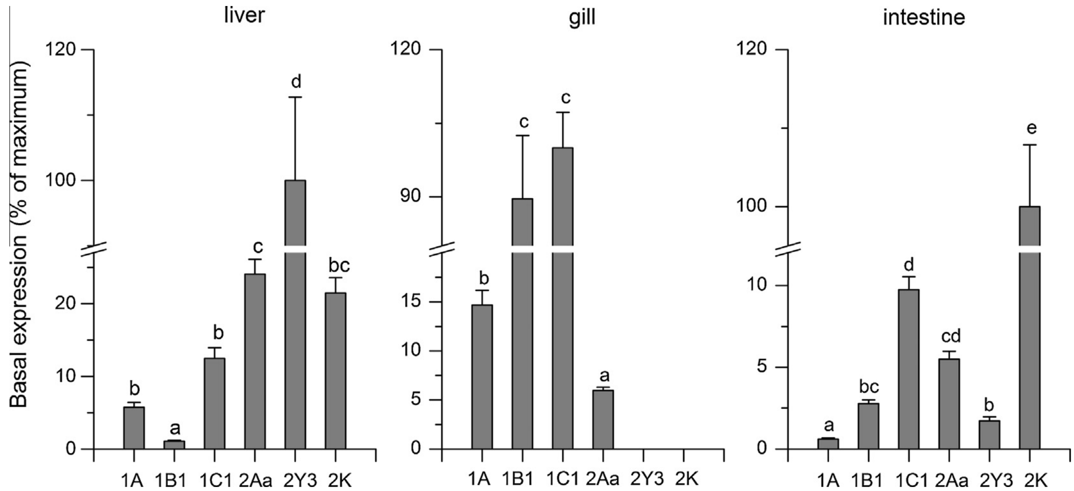

Fig. 3. Basal levels of CYP1and CYP2 transcript of rare minnow in a given tissue: liver, gill and intestine. Data are presented as a percentage of the highest CYP transcript in each tissue. Data are shown as means ± S.E. A statistical difference between groups at $p < 0.05$ ($n = 3$, ANOVA) is indicated by differences in the letters above the bars.

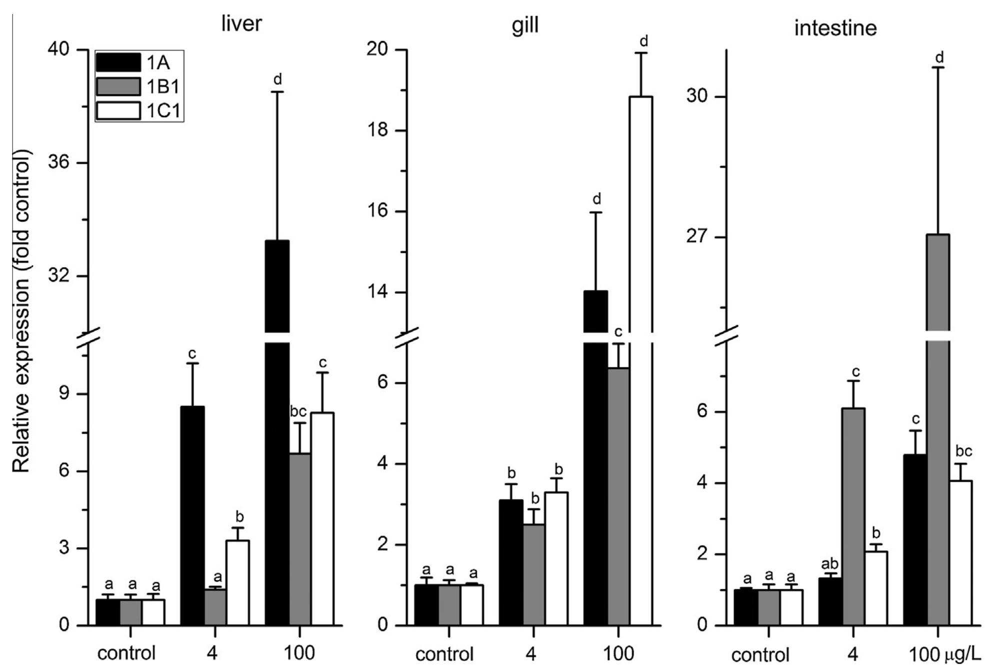

Fig. 4. Fold induction of CYP1A, 1B1, and 1C1 in rare minnow liver, gill, and intestine following BaP (4, 100 μg L^{-1}) exposure. Bars represent the relative mRNA expression in exposed fish versus control (acetone) fish. β-Actin was used for normalization. Values are presented as the mean ± S.E. A statistical difference between groups at $p < 0.05$ ($n = 3–5$, ANOVA) is indicated by differences in the letters above the bars.

minnow and zebrafish displays high degree of amino acid sequence similarity.

4.2. Basal expression of the CYP1 and CYP2 gene in adult rare minnow

CYP genes play an important role to regulate physiological functions. In this study, different tissue expression profiles of these CYP1 and 2s have been observed in rare minnow. The transcripts of the CYP1s were detected almost in all the tissues examined, and the basal expression patterns varied in the tissues, indicating that the three CYP1s have tissue-specific regulation and possibly different functions, which was similar to those previous studies in other fish (Jönsson et al., 2007; Zanette et al., 2009; Gao et al., 2011). Our results shows the highest and lowest levels of CYP1A mRNA expression were in the liver and brain, respectively, which largely agreed with previous studies with zebrafish (Jönsson et al., 2007), stickleback (Gao et al., 2011), and killifish (Zanette et al., 2009). However, in this study, the expression of CYP1B1 in rare minnow was high in the brain and low in the liver. This may due to the different endogenous functions of CYP1A and CYP1B1. CYP1A has been proved to play a role in metabolizing endogenous AHR ligands, while CYP1B1 can catalyze the formation of retinoic acid and may play a role in retinoic acid mediated patterning during embryogenesis (Gao et al., 2011). In previous studies, the transcripts of CYP1A and CYP1B1 in mammals and other fishes were detected in muscle or skin (Yengi et al., 2003; Lee et al., 2005; Erdoğan et al., 2011), whereas the transcripts of CYP1A and CYP1B1 in rare minnow were not detected (Fig. 2). The species differences may account for this conflict results. The level of CYP1C1 of rare minnow in muscle and skin were much higher than the other

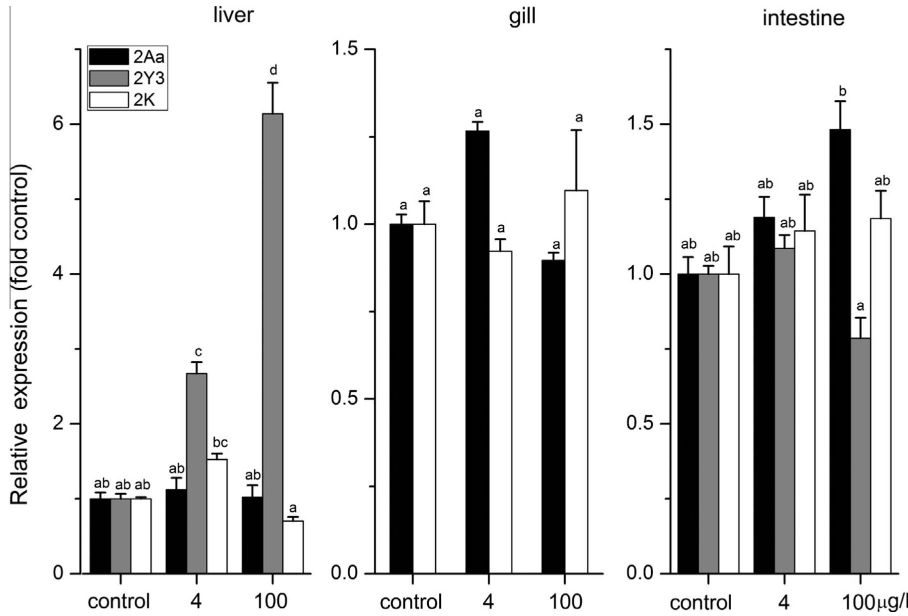

Fig. 5. Fold induction of CYP2Aa, 2Y3, and 2K in rare minnow liver, gill, and intestine following BaP (4, 100 μg L^{-1}) exposure. Bars represent the relative mRNA expression in exposed fish versus control (acetone) fish. β-Actin was used for normalization. Values are presented as the mean ± S.E. A statistical difference between groups at $p < 0.05$ ($n = 3–5$, ANOVA) is indicated by differences in the letters above the bars.

organs (Fig. 2). The high mRNA level of CYP1C1 in muscle and skin of rare minnow suggests that CYP1C1 may play an important role in xenobiotic metabolism.

For the CYP2s, the expression of CYP2K was detected only in three tissues (liver, kidney, and intestine), previous research has reported that the orthologous CYP2K6 transcript was only detected in liver and ovary of zebrafish (Wang-Buhler et al., 2005), and the CYP2K1 mRNA was mainly expressed in the liver and trunk kidney of rainbow trout (Buhler et al., 1994). Similar with CYP2K, the expression of CYP 2Y3 was observed only in liver, muscle, intestine, and spleen (Fig. 2). Interestingly, the CYP 2Aa was present in all the tissues analyzed similar with CYP 1s.

The expression of all six CYP genes was observed in liver, intestine and kidney, except no expression of CYP2Y3 in kidney, these tissues are all located in the abdominal cavity of rare minnow. This could be linked with the role of these tissues in nutrient uptake and processing of body waste products, e.g. detoxification of endogenous metabolites and food derived pollutants (Jönsson et al., 2007; Zanette et al., 2009).

4.3. Response of the CYP1s and CYP2s to BaP

CYP1 enzymes are prominent in metabolism of many toxicants including PAHs, and expression level is a major factor influencing the role of CYPs in substrate oxidation and effects *in vivo* (Jönsson et al., 2007). In the present study, all three CYP 1 genes transcription were significantly elevated in liver, gill and intestine of rare minnow exposed to BaP for 12 days (Fig. 4). Previous studies have observed the elevated expression of the CYP 1s (CYP1A, 1B1 and 1C1) by AHR agonist in various tissues of fish (Jönsson et al., 2007, 2010; Zanette et al., 2009; Gao et al., 2011; Dorrington et al., 2012). However, level of induction were significantly different among those fish and rare minnow, e.g. about 10-, 200-, and 400-fold for CYP1A, 1B1 and 1C1 in killifish liver (Zanette et al., 2009). This difference might due to different species, chemicals, and exposure methods. Since the chemical was administered via intraperitoneal injection to killifish and via water to rare minnow, thus the difference in induction may due to different bioavailability as they followed different exposure methods (Jönsson et al., 2010).

The BaP induction pattern of these CYP1s varied in the tissues of rare minnow, suggesting tissue-specific regulation and different function of these enzymes. Previous studies also showed different induction patterns of the CYP1s by AHR agonists (Zanette et al., 2009; Dorrington et al., 2012). It is widely held that fish CYP1s are regulated by AHR, ligand-activated AHR dimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT) and binds to xenobiotic response elements (XREs), causing the induction of CYP1 genes. The number of potential XREs upstream of the CYP1A genes is more than that in the CYP1B1 and CYP1Cs in zebrafish, the number and position of XREs in the CYP1 genes may influence the regulation of these genes (Zeruth and Pollenz, 2005; Jönsson et al., 2007). In addition, *in vitro* study results in MCF-7 cells indicated that the differential requirement for BRG-1 (a co-activator protein) may be a consequence of different nucleosomal configurations over the CYP1A1 and CYP1B1 genes, furthermore, maybe related to their different degrees of induction by dioxin (Taylor et al., 2009). Therefore, the different induction of the CYP1s by BaP in rare minnow may due to the differential requirement of the co-activators.

The CYP2 family is the largest and most diverse of the vertebrate CYPs and plays an important role in mammalian drug metabolism (Kirischian et al., 2011). In the present study, BaP significantly elevated the expression of CYP2Y3 in rare minnow liver (Fig. 5). Nonylphenol and bisphenol A exposure produced a significant reduction of CYP2Y3 in Atlantic cod (Olsvik et al., 2009), however, the regulating mechanism is unclear. Previous study showed zebrafish CYP2Y3 had a syntenic relationship to a cluster of CYP2 genes including CYP2S1 (Goldstone et al., 2010). Moreover, CYP2S1 is induced by AHR agonist in mammals (Saarikoski et al., 2005), which was consistent with our result of CYP2Y3 in rare minnow. Furthermore, to confirm the CYP2Y3 is regulated by AHR in fish, further studies should be done to discover whether there are some XREs in this gene like the CYP1A gene. Despite this, the CYP2Y3 gene may serve as a potential molecular biomarker of AHR agonist (e.g. BaP).

5. Conclusion

In summary, we identified and cloned the partial cDNAs of previous unreported CYP1B1, CYP1C1, CYP2Aa, CYP2Y3, and CYP2K in rare minnow. Basal expression of the CYP1s and the CYP2Aa were detected almost in all the tissues examined, while the expression of CYP2K and CYP2Y3 were only detected in several tissues of rare minnow. BaP significantly induced the expression of the CYP1s in all the tissues examined, as well as the CYP2Y3 in the liver. The basal and the induced expression varied in these tissues, indicating tissue-specific regulation and different function of these enzymes in rare minnow. The strong induction of CYP2Y3 mRNA level by BaP indicates that it may be a downstream gene of AHR and a potential molecular biomarker of AHR agonist. The responsiveness of these CYP genes in rare minnow means the fish is a suitable model to screen environmental AHR agonist (e.g. PAHs).

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